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(54) Title: PLANT EXTRACTS AND ANTI-CANCER MODALITIES INVOLVING THE SAME

(57) Abstract

Compositions comprising a water/organic solvent extract of clover from which aromatic chromophore containing compounds including the isoflavones genistein, daidzein, formononetin and biochanin and/or their glycosides have been removed, said extract having anti-cancer activity against one or more of the cell lines HL60, K562, LNCaP or HT29, optionally in association with one or more pharmaceutically acceptable carriers, excipients, auxiliaries, and/or diluents are described as are processes for the production of these compositions. Methods of treatment, prophylaxis, amelioration or defence against cancer are also disclosed.

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PLANT EXTRACTS AND ANTI-CANCER MODALITIES INVOLVING THE SAME

Technical Field

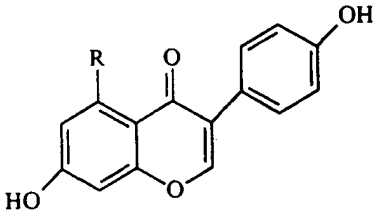
This invention relates to compositions comprising plant extracts, particularly from clover from which the isoflavones genistein, daidzein, formononetin and biochanin have been removed. More particularly, aromatic chromophore containing compounds including the aforementioned isoflavones are removed to provide extracts having anti-cancer activity. The invention also relates to therapeutic uses and the methods of treatment, particularly in the treatment of cancer.

Background of the Invention

Isoflavones have been extensively described in the scientific and patent literature as possessing a range of biological activities including oestrogenic and anti-cancer effects.

Naturally occurring isoflavones are found in plants such as legumes. These include soy, chick peas, lentils, beans (broad, haricot, kidney, lima, navy, etc), grams (Bengal, horse and green) and clovers. Soy and clover contain the highest levels of isoflavones.

Principal oestrogenic and anti-cancer isoflavones are genistein, daidzein, formononetin, and biochanin. In plants these compounds occur principally in the glycoside form bound to sugars such as glucose. The formulae of these isoflavones are as follows:



daidzein R = H
genistein R = OH

the structure of biochanin is the same as for genistein but with a 4'-methoxy group, and similarly formononetin has the same structure as daidzein, but with a 4'-methoxy group.

Isoflavone-containing plant extracts, such as from soy, are commercially available in some countries particularly as health supplements. Such extracts are specifically prepared for their isoflavone content, considerable effort being made to ensure the extracts contain maximum isoflavone content, the isoflavones being regarded as the biologically active species.

International Patent Application No. PCT/AU93/00230, incorporated herein by reference, describes isoflavone-containing extracts of a plant, such as legumes and clovers, and therapeutic uses for such isoflavone-containing extracts. The isoflavone extracts described therein specifically contain genistein, daidzein, formononetin and biochanin.

Clovers have been previously regarded as a herb or plant which may possess some anti-cancer activity. A range of compounds found in clovers have been shown to have some anti-cancer activity, including the aforementioned isoflavones and other isoflavones such as pratensin, and their appropriate glycosides, flavones such as quercetin (and aglucone forms such as kampferol), coumestens such as coumesterol, revesitrol, phytic acid and Vitamin A. These compounds are generally characterised by content of chromophores, particularly an aromatic chromophore, more particularly a phenolic ring component, or in the case of phytic acid, phosphate/phosphoric acid content.

A recent clinical finding described in the *Medical Journal of Australia* (Stephens, F. O. (1997) M. J. A., 167, 138-140) showed that an isoflavone extract prepared according to PCT/AU93/00230 produced major degenerative changes (including apoptosis) as shown by a prostatectomy specimen surgically removed from a patient with moderately high-grade adenocarcinoma. The patient received oral administration of the isoflavone-containing extract at a daily dose of 160 mg for seven days prior to surgery. The prostatectomy specimen showed a moderately high-grade adenocarcinoma with patchy microvaculation and

prominent apoptosis, while no change was seen in normal prostate cells. The degenerative changes in the prostatecomy specimen, especially the apoptosis, were indicative of androgen deprivation and typical of a response to oestrogen therapy. This finding provided direct support for the oestrogen-like activity of the isoflavones genistein, daidzein, formononetin and biochanin exerting a positive therapeutic treatment in prostate cancer.

The inventors have surprisingly found that extracts of clover, from which aromatic chromophore containing compounds including genistein, daidzein, formononetin and biochanin have been removed, have significant anti-cancer activity. This finding was contrary to all predictions, particularly given the absence of genistein, daidzein, formononetin and biochanin, hitherto regarded as the principal active anti-cancer agents in clover.

Summary of the Invention

In its broadest aspect this invention is concerned with a composition comprising an extract of clover from which the isoflavones genistein, daidzein, formononetin and biochanin and/or their relevant glycosides (for convenience hereinafter referred to as GDFB) have been removed. More particularly, aromatic chromophore containing compounds including the aforementioned isoflavones and their glycosides are removed to give the extracts of the invention.

In another aspect this invention is concerned with a composition comprising a water/organic solvent extract of clover from which aromatic chromophore containing compounds including the isoflavones genistein, daidzein, formononetin and biochanin and/or their glycosides have been removed, said extract having anti-cancer activity against one or more of the cell lines HL60, K562, LNCaP or HT29, optionally in association with one or more more pharmaceutically acceptable carriers, excipients, auxiliaries, and/or diluents.

In accordance with another aspect of this invention there is provided a process for the production of a composition comprising an extract of clover which process comprises

extracting the clover with a mixture of water/organic solvent, recovering the organic solvent component from residual undissolved plant material, and thereafter removing aromatic chromophore containing compounds including daidzein, formononetin and biochanin and/or their glycosides to form an extract having anti-cancer activity against one or more of the cell lines HL60, K562, LNCaP or HT29, and optionally formulating the extract with one or more pharmaceutically acceptable carriers, excipients, auxiliaries, and/or diluents.

In another aspect of this invention there is provided a composition when prepared according to the above process.

In a further aspect of this invention there is provided an extract of clover from which aromatic chromophore containing compounds including genistein, daidzein, formononetin and biochanin have been removed, for the manufacture of a medicament for the treatment, prophylaxis, amelioration or defence against cancer.

In accordance with another aspect of this invention there is provided a method for the treatment of prophylaxis, amelioration or defence against cancer which comprises administering to a subject in need of such a treatment an effective amount of a composition as described herein.

Detailed description of the Invention

In its broadest aspect this invention is concerned with a composition comprising an extract of clover from which aromatic chromophore containing compounds including the isoflavones genistein, daidzein, formononetin and biochanin (GDFB) and/or their relevant glycosides have been removed. Such compositions may be in the form of pharmaceutical compositions, in association with one or more pharmaceutically acceptable carriers, excipients, auxiliaries and/or diluents. Compositions according to the invention have potent anti-cancer activity thereby allowing for the prophylaxis, amelioration, prevention and/or treatment of cancer.

Cancer is a major cause of death and of morbidity in the human population, particularly in the middle aged and elderly, but also across the whole human population including children. By way of example, breast cancer is a major cause of cancer in women, particularly after menopause (Bonett *et al*, (1992) *Eur. J. Cancer*, 1926). Prostate cancer is now the second most common cause of cancer death in men in the United States after cardiovascular disease, where on average one in ten men may be expected to develop prostate cancer, with an average loss of life of nine years after cancer development. As many as 1 in 4 men may develop prostatic enlargement which may lead to prostate cancer.

Bowel cancer or colon cancer is a significant cancer, particularly in societies consuming a "Western diet". These cancers may have some association with a diet which is relatively high in unsaturated fat content and low in complex carbohydrates. Bowel cancer metastis are often very refractive to anti-cancer therapies, thus causing bowel cancer to be a major cause of morbidity.

Chemotherapy for the treatment of cancer is harsh, often generally being cytotoxic and affecting fast growing cells such as those of the intestinal region, and hair cells. Unpleasant side effects often include nausea, loss of taste, lethargy, hair loss, loss of libido, and the like. Anti-cancer therapeutic agents are generally costly and in some cases may only be administered by intravenous infusion.

The compositions of the present invention provide anti-cancer activity for the treatment, prophylaxis, amelioration and/or prevention of cancer, whilst overcoming one or more disadvantages of chemotherapeutic agents available for the treatment of cancer.

In accordance with an aspect of this invention there is provided a composition which comprises a water/organic solvent extract of clover from which aromatic chromophore containing compounds including the isoflavones genistein, daidzein, formononetin and biochanin and/or their glycosides have been removed, optionally in association with one or more pharmaceutically acceptable carriers, excipients, auxiliaries, and/or diluents.

The compositions of the present invention may be prepared from any clover (*Trifolium*) including red clover (*T. pratense*), such as Red Quin, Pawera, Pac 19, Quine Queli, Renegade, Hamua, Asterid and Colenso, subterranean clover (*T. subterranean*), white clover (*T. repens*), or any clover related species.

Plant material may be dried, and may be chopped or otherwise comminuted by methods well known in the art prior to an extract being prepared thereof. The extract may be made from one or more species of clover

The compositions of the invention comprise a water/organic solvent extract of clover from which aromatic chromophore containing compounds including genistein, daidzein, biochanin and formononetin and/or their glycosides are specifically removed. The ratio of water to organic solvent is generally in the order of 0.5% to 70% v/v water organic solvent, preferably from 1% to 50% organic solvent. The organic solvent is preferably a C_{1-4} organic solvent (such as methanol, ethanol, propanol, propylene glycol, erythrite, butanol, butanediol, acetonitrile, ethyleneglycol, glycidol, glycerol dihydroxyacetone or acetone). The extract in this regard is prepared by exposing the plant material to the water/organic solvent mix. The exposure time in general terms is indirectly proportional to the temperature of the mixture. The temperature of the mix may range, for example, from an ambient temperature to boiling temperature. Exposure time may be between one hour to several weeks. One convenient extraction period is twenty four hours at 90°C. The extract is separated from undissolved plant material and the solvent removed by distillation, rotary evaporation, or other standard procedures for solvent removal. The distillation residues containing water soluble and non-water soluble components and water are preferably extracted with non-water miscible organic solvent or non-polar solvent (such as petroleum ether, pentane, hexane, heptane, octane, benzene or toluene) and the aqueous phase discarded. The isoflavones genistein, daidzein, formononetin and biochanin, or their glycosides, and other aromatic chromophore containing compounds may be removed at this stage to give the final extract, or alternatively the organic solvent may be removed to give

a residue which may be dried and from which the aromatic chromophore containing compounds may be subsequently removed.

Compounds having aromatic chromophore content including the isoflavones genistein, daidzein, formononetin, biochanin flavones including pratensin, reversitrol and Vitamin A are removed from the extract to give a final plant extract as utilised herein by standard procedures. Examples include chromotagraphic techniques, such as preparative high performance liquid chromatography (HPLC) using UV detection, and reverse phase HPLC using UV detection. Aromatic chromophore containing compounds show a characteristic UV absorbence between about 254 and 300nm as does Vitamin A. This allows these compounds to be readily removed giving the extract of the invention. Aromatic chromophore containing compounds including genistein, daidzein, formononetin and biochanin and/or their relevant glycosides are identified by UV analysis and specifically removed. Eluates from which aromatic chromophore containing compounds including genistein, daidzein, formononetin and biochanin and/or their glycosides have been removed are then pooled and may be concentrated, (for example, by solvent removal and drying to give a power) with subsequent formulation into pharmaceutically acceptable compositions. Examples of chromatographic media include inorganic materials (such as porous silica, controlled poreglass hydroxy apatite, fluorapatite, aluminium oxide), composite materials (such as coated silica, coated polystyrene) and synthetic polymers (polyacrylamide, polymethacrylate, and polystyrene) and reverse phase HPLC matrixes including C_8-C_{18} columns. The solvent phase for chromotographic separation may be an organic solvent such as methanol, ethanol, propanol, butanol, pentanol, acetone, butanone, chloroform, dichloromethane, dichloroethane, dichlorobutane, ethylacetate, ether or dimethyl sulphoxide, which may be used to dissolve the extract prior to separation. Other procedures for specifically removing isoflavones include differential extraction with organic solvents, based on the differing solubility of aromatic chromophore containing compounds in certain organic solvents (see, for example, *Burdick and Jackson Solvent Guide*, Third Edition, Burdick and Jackson Laboratories, Muskegon, MI, 1990), such as non-water miscible organic solvents.

In a further aspect of this invention there is provided a process for the production of a composition comprising an extract of clover which process comprises extracting the clover with a mixture of water/organic solvent, recovering the organic solvent component from residual undissolved plant material, and thereafter removing aromatic chromophore containing compounds including daidzein, formononetin and biochanin and/or their glycosides to form an extract having anti-cancer activity against one or more of the cell lines HL60, K562, LNCaP or HT29, and optionally formulating the extract with one or more pharmaceutically acceptable carriers, excipients, auxiliaries, and/or diluents.

As set forth above compositions according to the present invention may include one or more pharmaceutically acceptable carriers. The carriers are selected so as to be acceptable in the sense of being ingredients in the composition and must not be deleterious to the patient. The carriers may be solid or a liquid, or both, and may be formulated with the extract as a unit-dose, for example a tablet, which may contain from 0.5% to 59% by weight of the active compound or up to 100% by weight to the active compound. Compositions may be prepared by any of the well known techniques of pharmacy, for example admixing the components, optionally including excipients, diluents (for example water) and auxiliaries as are well known in the pharmaceutical field.

The compositions according to the invention may include one or more active agents, such as vitamins (for example, Vitamin A, Vitamin B group, Vitamin C, Vitamin D, Vitamin E and Vitamin K), minerals (for example, magnesium, iron, zinc, calcium and manganese in the form of pharmaceutically acceptable salts), chemotherapy agents including anti-multi-drug resistant compounds (for example, alkylating agents, anti-metabolites, vinca alkaloids, antibiotic cytotoxics, hormonal antineoplastic agents, and synthetic cytotoxics), immune stimulators (for example, any interferon, interleukin, and growth hormones/growth factors), and anti-oxidants.

The compositions of the invention include those suitable for oral, rectal, optical, buccal (for example sublingual), parental (for example subcutaneous, intramuscular, intradermal and

intravenous) and transdermal administration. The most suitable route in any given case will depend on the nature and severity of the condition being treated and the state of the patient.

Compositions suitable for oral administration may be presented in discrete units, such as capsules, cachets, lozenges, or tablets, each containing a predetermined amount of the extract; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. Such compositions may be prepared by any suitable method of pharmacy which includes the step of bringing into association the active compound and one or more suitable carriers (which may contain one or more accessory ingredients as noted above). In general the compositions of the invention are prepared by uniformly and intimately admixing the extract with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the resulting mixture. For example, a tablet may be prepared by comprising or moulding a powder or granules containing the extract, optionally with one or more accessory ingredients. Compressed tables may be prepared by compressing in a suitable machine, the extracts in the form of a powder or granules optionally mixed with a binder, lubricant, inert diluents, and/or surface active/dispersing agent(s). Moulded tablets may be made by moulding, in a suitable machine, the powdered compound moistened with an inert liquid binder.

Suitable carriers may be fillers, such as sugars, for example lactose, saccharose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, for example tricalcium phosphate or calcium hydrogen phosphate, and also binders, such as starch pastes using, for example, corn, wheat, rice or potato starch, gelatin, tragacanth, methylceullose and/or polyvinylpyrrolidone, and, if desired, disintegrators, such as the above-mentioned starches, also carboxymethyl starch, cross linked polyvinyl pyrrolidone, agar or alginic acid or a salt thereof, such as sodium alginate. Excipients may be flow conditioners and lubricants, for example silicic acid, talc, stearic acid or salts thereof, such as magnesium or calcium stearate, and/or polyethylene glycol. Dragée cores are provided with suitable, optionally enteric, coatings, there being used, *inter alia*, concentrated sugar solutions which may comprise gum arabic, talc, polyvinylpyrrolidone, polyethylene glycol and/or titanium

dioxide, or coating solutions in suitable organic solvents or solvent mixtures, or, for the preparation of enteric coatings, solutions of suitable cellulose preparations, such as acetylcellulose phthalate or hydroxypropylmethylcellulose phthalate. Dyes or pigments may be added to the tablets or dragée coatings, for example for identification purposes or to indicate different doses of active ingredients.

Other orally administrable pharmaceutical compositions are dry-filled capsules made, for example, of gelatin, and soft, sealed capsules made of gelatin and a plasticiser, such as glycerol or sorbitol. The dry-filled capsules may comprise the extracts in the form of granules, for example in admixture with fillers, such as lactose, binders, such as starches, and/or glicants, such as talc or magnesium stearate, and, where appropriate, stabilisers. In soft capsules, the extract is preferably dissolved or suspended in suitable liquids, such as fatty oils, paraffin oil or liquid polyethylene glycols, to which stabilisers may also be added.

Formulations suitable for buccal (sublingual) administration include lozenges comprising the extracts in a flavoured base, usually sucrose and acacia or tragacanth; and pastilles comprising the compound in an inert base such as gelatin and glycerin or sucrose and acacia.

Compositions of the present invention suitable for parenteral administration conveniently comprise sterile aqueous preparations of the extracts, which preparations are preferably isotonic with the blood of the intended recipient. These preparations are preferably administered intravenously, although administration may also be effected by means of subcutaneous, intramuscular, or intradermal injection. Suitable compositions include water soluble extracts and also suspensions of the active ingredient, such as corresponding oily injection suspensions, there being used suitable lipophilic solvents or vehicles, such as fatty oils, for example sesame oil, or synthetic fatty acid esters, for example ethyl oleate or triglycerides, or aqueous injection suspensions comprising viscosity-increasing substances, for example sodium carboxymethylcellulose, sorbitol and/or dextran, and, where appropriate, also stabilisers. As an example compositions may conveniently be prepared by admixing the extracts with water or a glycine buffer and rendering the resulting solution

sterile and isotonic with the blood. Injectable formulations according to the invention may contain from 0.1% to 60% w/v of the extract and may, for example, be administered at a rate of 0.1 ml/minute/kg.

Formulations suitable for rectal administration are preferably presented as unit dose suppositories. These may be prepared by admixing the extracts with one or more conventional solid carriers, for example cocoa butter, and then shaping the resulting mixture.

Compositions suitable for topical administration to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers which may be used include petroleum jelly, lanoline, polyethylene glycols, alcohols, and a combination of two or more thereof. The extract is generally present at a concentration of from 0.1% to 30% weight/weight, for example from 0.5% to 10% weight/weight.

Compositions suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Such patches may contain the extracts in an optionally buffered aqueous solution.

Compositions suitable for transdermal administration may also be delivered by iontophoresis (see, for example, *Pharmaceutical Research 3* (6), 318 (1986)) and typically take the form of an optionally buffered aqueous solution of the extracts. Such compositions may, for example, contain citrate or bis/tris buffer (pH 6) or ethanol/water, with for example .05% to 30% w/w extract.

Compositions may be prepared in a manner, and in a form/amount as is conventionally practised. See for example, Goodman & Gillman, *The Pharmalogical Basis of Therapeutics* (7th Edition, 1985) and *Remington's Pharmaceutical Science* (Mack Publishing Company,

10th Edition), both of which are incorporated herein by reference. Compositions may contain, for example, from 0.1 mg to 2 g extract, such as 0.1 mg to 200 mg.

The extracts from which aromatic chromophore containing compounds including genistein, daidzein, biochanin or formononetin have been removed may be in the form of a powder, a slurry, in aqueous solution, particulate form, or dissolved in an organic solvent (such as methanol, ethanol, ethylacetate or dimethyl sulphoxide).

What constitutes an effective amount of the compositions of the present invention will depend upon a number of factors, such as specific mode of administration, the cancer being treated, the condition of the patient and the judgement of the health care giver. Examples of dosages of extracts are about 0.1 mg to about 200 mg per day, such as in the order of 1.5 mg/kg (body weight)/day.

In cancer cells, the compositions of the present invention induce one or more effects of inhibition of cell proliferation, induction of cell differentiation, induction of apoptosis (programmed cell death), DNA fragmentation or cell cycle blocking which all represent anti-cancer activity. As a consequence, the compositions of the present invention have wide ranging activity against cancer cells and are accordingly effective in the treatment, prophylaxis, amelioration, defence against and/or prevention of cancers including benign prostatic hypertrophy, prostatic cancer, breast cancer, uterine cancer, leukaemia, ovarian cancer, endometrial cancer, cervical cancer, colon (large bowel) cancer, testicular cancer, Hodgkin's disease, lymphoma, rhabdo sarcoma, neuroblastoma, pancreatic cancer, lung cancer, brain tumour, skin cancer, stomach (gastric) cancer, oral cancer, liver cancer, laryngeal cancer, bladder cancer, thyroid cancer, and nasopharyngeal carcinoma. As the compositions of the present invention are free from side effects in all tests carried out to date, and given their natural product origin, they are especially suitable as prophylactics in the defence against cancer. In pre- and post-menopausal women, and in males, for example over the age of forty, the defence against highly prevalent cancers, such as breast cancer in women and prostate cancer in men, is highly advantageous from a number of perspectives.

These include decreased mortality and morbidity, reduction in health care expenses, general patient well being and the like. The prevention or control of cancer, such as cancer of the prostate, breast, colon or leukaemia may be effected by daily administration of the compositions of the invention, such as by oral administration

This invention will now be described with reference to the following non-limiting examples.

Example 1

Red clover is harvested and dried by either sun-drying or applied heat. The material is optionally chaffed and extracted with a mixture of organic solvent and water. Sixty percent ethanol in water is used. Extraction is carried out at 90°C for twenty four hours. The supernatant is separated from undissolved plant material, and solvent removed by distillation. The residue comprising water, water soluble components and non-water soluble components is extracted with a non water-miscible organic solvent (such as petroleum ether), followed by removal of the aqueous phase containing water soluble components. Removal of organic solvent by distillation (or drying under vacuum) gives a tar-like residue, which can be dried to give a powder, or which can be dissolved in organic solvent to give an isoflavone containing extract.

In the same manner as above, an isoflavone-containing extract is prepared from soy hypocotyls and cotyledons.

Example 2

Separation of primary isoflavones using chromatographic techniques

A red clover extract according to Example 1 is dissolved in either methanol, ethanol, propanol, butanol, pentanol, acetone, butanone, chloroform, dichloromethane, dichloroethane, dichlorobutane, ethyl acetate, ether or dimethyl sulphoxide. The supernatant is injected into a preparative HPLC system with UV detection. From the known retention times of GDBF these are removed and the remaining peaks combined to give isoflavone free extract. The solvent is removed by evaporation or distillation, and the final

extract dissolved in a small amount of ethanol or dimethyl sulphoxide (DMSO), or dried to give a powder, optionally in association with one or more pharmaceutically acceptable carriers.

Example 3

Method for solvent extraction of aromatic chromopore containing compounds

Fifty kilograms of a red clover according to Example 1 is placed in a 2000 L stainless steel drum. It is extracted with 1500 L acetone:hexane (3:7) mixed solvent with stirring at ambient temperature for between four to twenty hours.

The extract is dried under vacuum (-40 KPa to -97 KPa) at 30°C to 80°C.

The dried extract is placed in a 200 L stainless steel drum and washed with 100 L non-polar organic solvent (such as petroleum ether, pentane, hexane, heptane, octane, benzene or toluene) for four to ten times. The residue solid material is then produced after being dried under vacuum. Solvent was removed under vacuum (-40KPa to -97 KPa) at 30°C to 80°C.

The final product obtained at step 4 was dissolved in dimethyl sulphoxide (20 mg/ml to 100 mg/ml) for pharmacological testing.

Example 4

Anti-cancer activity or isoflavone free cell extracts

Cancer cell lines are screened for anti-cancer activity. Cell lines are chosen to reflect most prevalent types of cancer in the human population, namely breast cancer, colon cancer, prostate cancer and leukaemia. The cell lines screened included leukaemic cell lines HL60 and K562, prostate cancer cell lines LNCaP (androgen receptor positive) and DU145 (androgen receptor negative), and colon cancer cell line HT29.

Anti-cancer activity was assessed by culturing cells in culture medium in the presence, or absence, of predetermined amounts of candidate anti-cancer agents and appropriate controls.

Anti-cancer activity was measured by assessing inhibition of cell proliferation, cell cycle analysis, apoptosis, differentiation, DNA fragmentation and growth inhibition. Cell proliferation was measured by the incorporation of (^{3}H)-thymidine using the standard MTT assay (Marks *et al*, (1992) *Leukaemia Research*, 16:1165-1173). Cell cycle, apoptosis and DNA fragmentation are determined by flow cytometry (McCloskey *et al* (1994) *Clinical Immunology and Immunopathology*, 71:14-15).

Agents tested are genistein (A1), an isoflavone-containing extract of red clover (A2) according to Example 1, isoflavone free extracts of red clover and soy prepared according to Examples 2 (A3) and 3 (A4), and water soluble components of red clover prepared according to Example 1 (A5). Concentrations of agents tested are 0, 10, 20 and 40 μg/ml.

Controls which contained either only medium or medium and the carrier used for the agents, had no effect as expected. An anti-cancer effect is observed for A1, A2, A3 and A4, but not A5. A3 and A4 are much more potent than the other agents tested. For example, in one experiment, using the cell line LNCaP at 40 μg/ml agent (calculated on a weight/weight basis), A3 was between five and ten times more active than A1 and A2, showing 95% cell death and substantial cellular irregularities in the remaining cells indicative of an anti-cancer effect. This was a most surprising result as A3 and A4 were not expected to have anti-cancer activity. The same results were observed for the breast and leukaemia cell lines.

Example 5

Treatment of a sixty two year old male diagnosed with prostate cancer

A sixty two year old healthy male was diagnosed with a moderately high-grade adenocarcinoma of the prostate after a digital rectal exam found an enlarged prostate gland, and pathology results showed a raised PSA (prostate specific antigen 14 ug/L). Further, a multiple biopsy showed that two of six transrectal needle biopsy specimens revealed low-grade carcinoma.

The patient declined radiotherapy and elected to take an isoflavone free composition produced according to Example 3, at a dose of 200mg (5 x 40mg tablets) per day for fourteen days prior to a radical prostatectomy. The resected specimen showed prominent apoptosis, typical of a response to anti-cancer therapy and/or adrogen deprivation, and suggestive of tumour regression. There were no adverse side effecs or changes seen in normal prostate cells. A three-month review found that his PSA level was found to be less than 0.1ug/L.

Example 6

Patient AB, a 48 year old male suffered from metastatic cancer in the liver, derived from a primary esophageal adenocarcinoma. The metastatic liver cancer was regarded by a biopsy to be highly aggressive. The patient initially received chemotherapy in the form of 5-fluoro uracil which reduced liver tumour load by 50%. The patient subsequently received the isoflavone-free extract according to Example 3 at a dose of 200mg per day. Liver function returned to normal having been grossly abnormal and the patient was considered to be receiving treatment/amelioration from his cancerous condition giving him a stable/remission outcome.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising" or the term "includes" or variations thereof, will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers. In this regard, in construing the claim scope, an embodiment where one or more features is added to any of claims is to be regarded as within the scope of the invention given that the essential features of the invention as claimed are included in such an embodiment.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications which fall within its spirit

and scope. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

References referred to herein are to be regarded as being incorporated by reference.

CLAIMS

1. A composition comprising a water/organic solvent extract of clover from which aromatic chromophore containing compounds including the isoflavones genistein, daidzein, formononetin and biochanin and/or their glycosides have been removed, said extract having anti-cancer activity against one or more of the cell lines HL60, K562, LNCaP or HT29, optionally in association with one or more more pharmaceutically acceptable carriers, excipients, auxiliaries, and/or diluents.

2. A composition according to claim 1 wherein the water/organic solvent extract is a water/alcohol extract.

3. A composition according to claim 1 wherein said clover includes red clover, subterranean clover or white clover.

4. A composition according to claim 3 wherein said clover is red clover including Red Quin, Pawera, Pac 19, Quine Queli, Renegade, Hamua, Asterid and Colenso.

5. A composition according to claim 1 which includes one or more active agents including vitamins, minerals, chemotherapy agents, immune stimulators and/or anti-oxidants.

6. A process for the production of a composition comprising an extract of clover which process comprises extracting the clover with a mixture of water/organic solvent, recovering the organic solvent component from residual undissolved plant material, and thereafter removing aromatic chromophore containing compounds including daidzein, formononetin and biochanin and/or their glycosides to form an extract having anti-cancer activity against one or more of the cell lines HL60, K562, LNCaP or HT29, and optionally formulating the extract with one or more pharmaceutically acceptable carriers, excipients, auxiliaries, and/or diluents.

7. A process according to claim 6 wherein the aromatic chromophore containing compounds are removed by HPLC on a reverse phase column.

8. A process according to claim 6 wherein the aromatic chromophore containing compounds are removed by differential solvent extraction.

9. A composition when prepared according to any of claims 6 to 8.

10. A method for the treatment, prophylaxis, amelioration or defence against cancer which comprises administering to a subject in need of such a treatment an effective amount of a composition as defined in any of claims 1 to 5.

11. A method according to claim 10 wherein said cancer is selected from beast cancer, prostate cancer including androgen dependent and/or androgen independent prostate cancer, leukaemia, colon cancer, uterine cancer, ovarian cancer, endometrial cancer, cervical cancer, colon (large bowel) cancer, testicular cancer, Hodgkin's disease, lymphoma, rhabdo sarcoma, neuroblastoma, pancreatic cancer, lung cancer, brain tumour, skin cancer, stomach (gastric) cancer, oral cancer, liver cancer, laryngeal cancer, bladder cancer, thyroid cancer, and nasopharyngeal carcinoma.

12. An extract of clover from which the isoflavones genistein, daidzein, formononetin and biochanin and/or their glycosides have been removed.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/00111

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: A61K 35/78

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: A61K 35/78

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT)
JAPIO) Clover or trifolium and extract
Medline)
CAS : Clover or trifolium and extract and cancer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Patent Abstracts of Japan, C-265, page 15 JP 59-175496 A (OOSAKA YAKUHIN KENKYUSHO KK) 4 October 1984 abstract	12
X	Patent Abstracts of Japan, C-442, page 66 JP 62-63581 A (COSMO CO LTD) 20 March 1987 abstract	12
X	Patent Abstracts of Japan, C-447, page 60, JP 62-83848 A (RIYOUSHIYOKU KENKYUKAI) 17 April 1987 abstract	12

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 April 1999	13 APR 1999
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929	Authorized officer TAMARA NIZNIK Telephone No.: (02) 6283 2422

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/00111

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB 2136812 A (OSAKA CHEMICAL LABORATORY CO LTD (JAPAN)) 26 September 1984 see page 1, lines 53-57, Examples 3, 4, 8	12
X	US 4140805 A (EDWARDS et al) 20 February 1979 see whole document	12
A	WO 93/23069 A (KELLY, Graham E) 25 November 1993 see whole document	
A	AU 40034/97 A (NOVOGEN RESEARCH PTY LTD) 14 May 1998 see whole document	
X	Derwent Abstract Accession No: 19629 E/10, Class B04 (B07), SU 833253 A (LATV CARDIOLOGY RES INST) 31 May 1981	12
A	Derwent Abstract Accession No: 97-279357/25, Class B04, RU 2068269 A (PYATIGORSK PHARM INST) 27 October 1996	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU 99/00111

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
JP	59-175496	AU	23057/84	DE	3400258	FR	2543144
		GB	2136812	US	4594412		
JP	62-63581	NONE					
JP	62-83848	NONE					
GB	2136812	AU	23057/84	DE	3400258	FR	2543144
		JP	59175496	US	4594412		
US	4140805	NONE					
WO	93/23069	AU	40525/93	BR	9306393	EP	656786
		NO	944435	NZ	252051	US	5830887
AU	40034/97	WO	98/08503	AU	2039/96		
SU	833253	NONE					
RU	2068269	NONE					

END OF ANNEX